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Advancing Immunomodulation by *In Vivo* Antigen Delivery to DEC205 and Other Cell Surface Molecules Using Recombinant Chimeric Antibodies

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Abstract

A targeted delivery of defined antigens *in vivo* allows for the probing of relevant functions of the immune system. Recombinant chimeric antibodies, produced by genetically modifying original monoclonal antibodies specific for molecules expressed on dendritic cells and other immune cells, have paved the way for the development of such strategies and have become reliable tools for achieving a specific immunomodulation. These antibodies have proven important in both basic research and clinical applications, extending data obtained in disease models of autoimmunity and cancer. Here we will describe the advances gained from the experimental and therapeutic strategies based on the targeting of the specific antigens by recombinant chimeric antibodies to the multilectin receptor DEC205 and other cell surface molecules.

Keywords

Recombinant Chimeric Antibody; Immunotherapy; Immunomodulation; Autoimmunity; Cancer; DEC205

Defined Delivery of Peptide Antigens to DCs

Conventional dendritic cells (cDCs or DCs) play integral roles in both the innate and adaptive immune responses due to their unique capacities to uptake, process, and present multiple foreign and self-antigens to T cells. Therefore, harnessing these functions of DCs by applying the methods of defined antigen delivery proves important in both basic scientific research and therapy for patients. The concept of delivering defined peptide antigens to DCs in order to promote desired cognate T cell responses was first based on early observations showing DCs' efficient uptake and processing of the immunoglobulin specifically binding to these cells [1]. However, to obtain specific T cell responses, the cognate T cell epitopes were subsequently introduced to original monoclonal antibodies specific for molecules expressed

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on the surfaces of DCs and their subsets. This can be achieved by chemically-coupling such antigens to antibody molecules, as well as by genetically fusing the specific antigens within recombinant antibodies. Other options include formation of single-chain fragment variable region (scFv) molecules [2–5]. Additionally, in a chimeric antibody design, the species-specific constant regions are used to minimize off-target effects. Therefore, among multiple options, recombinant chimeric antibodies may be more favorable in certain contexts, as they can be readily modified based on the desired solubility and specific molecular interaction capacities for *in vivo* administration in the organism of choice. Additionally, production allows for known numbers of antigenic peptides to be included in the reagent construct as well as for the minimization of contamination by endotoxin, rendering these reagents ideal for basic science and clinical applications [2, 6].

The evolutionary conservation of CD11c$^+$ DCs, plasmacytoid DCs (pDCs), and their development-governing transcription factors provides scientific justification for murine studies including those employing antigen targeting by recombinant chimeric antibodies [2, 7–9]. Briefly, the CD11c$^+$ DCs are subdivided into the DC1 and DC2 developmental lineages governed by the transcription factors IRF8/Batf3 and IRF4/Notch2, respectively. Murine DC1s are characterized by their expression of XCR1, and some also express CD8a, DEC205, BTLA, Langerin, Treml4, and Clec9a. In contrast, murine DC2s are generally characterized by their expression of CD172a (SIRPa) and additional expression of DCIR2 and CD11b, while pDCs are characterized by expression of DC-SIGN, Siglec-H, B220, and Ly6c [2, 8–11]. Whereas DC2s can preferentially promote T helper 2 (Th2), T helper 17 (Th17), and follicular helper T (Tfh) cell differentiation, DC1s have crucial roles in the cross-priming of CD8$^+$ T cells and the priming of CD4$^+$ T helper 1 (Th1) cells, as well as in the induction of CD4$^+$CD25$^+$Foxp3$^+$ peripheral regulatory T (pTreg) cells [2, 10, 12, 13]. Importantly, human DC1s, defined as CD141$^+$ (BDCA-3$^+$) XCR1$^+$ BTLA$^+$, and human DC2s, defined as CD1c$^+$ CD172a$^+$ CD11b$^+$, as well as BDCA-2$^+$ neuropiiin$^+$ pDCs, share many developmental, phenotypical, and functional similarities with their murine counterparts [7, 14–19].

DEC205 and the First Recombinant Chimeric Antibodies

The first recombinant chimeric antibodies that were initially designed to faithfully deliver defined peptide antigens to DCs targeted the endocytic receptor DEC205 (CD205, LY75) [20, 21]. DEC205 is expressed at high levels on murine DC1s [22]. Antigens targeted to DEC205 can be presented both in the context of MHCI and MHCII leading to very efficient cross priming of CD8$^+$ T cells and also activation of CD4$^+$ T cells. Although an activation of CD4$^+$ T cells by DC1s appears as less efficient than that mediated by DC2s, DC1s nevertheless have central roles in the promotion and maintenance of both immunogenic and tolerogenic CD4$^+$ T cell responses [2, 8–10, 23, 24].

The basic design of the anti-DEC205 chimeric antibody is comprised of the variable (V) regions specific for DEC205 that were cloned from the original hybridomas producing the rat anti-mouse antibody (NLDC-145). These V regions were genetically combined with modified murine lgG1 constant regions containing additional mutations to ensure minimal non-specific interactions with host cells *in vivo* [20]. Importantly, the C-termini of the

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constant regions of such DEC205-specific chimeric antibodies may be genetically fused with a defined antigen of choice. The entire recombinant chimeric antibody molecule is produced in a eukaryotic *in vitro* expression system [20], The first antigens that were included in this chimeric antibody construct were peptides: hen egg lysozyme ($HEL_{46–61}$) (anti-DEC205-HEL) and myelin oligodendrocyte glycoprotein ($MOG_{35–55}$) (anti-DEC205-MOG) [20, 21]. The administration of these novel chimeric antibodies demonstrated that defined antigen may be presented by DCs to cognate $CD4^+$ T cells *in vivo* and also revealed that, under “steady state” (non-inflammatory) conditions, presentation by DCs of such targeted antigens induced T cell tolerance in the periphery [20]. Particularly, experiments using anti-DEC205-MOG demonstrated the potential therapeutic benefits, as its administration blocked autoimmune responses and disease symptoms of experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS) [21]. Furthermore, by using MHCI-restricted and cross-presented antigens, subsequent experiments extended the initially established tolerogenic functions of the $DEC205^+$ DCs to the induction of tolerance among $CD8^+$ T cells [25], The effectiveness of targeting antigens to DEC205 to promote antigen-specific immune tolerance and to ameliorate disease severity was subsequently extended to other models of autoimmune disease, including diabetes, inflammatory bowel disease (IBD), and arthritis, as well as to a model of graft-versus-host disease [Figure 1] [26–31].

Further, the targeting of antigens to DEC205 has also been successfully applied as a promising vaccine approach in both infectious and tumor models. For example, intranasal administration of the adjuvant polyinosinic:polycytidylic acid (Poly(l:C)) together with recombinant anti-DEC205 antibodies targeting the Yersinia pestis virulence protein LcrV to DEC205-expressing cells induced IgG and IgA antibodies as well as IFNy-secreting $CD4^+$ T cells in the lung [32, 33]. Moreover, in the presence of anti-CD40 adjuvant, administration of recombinant anti-DEC205-HIVgag-p24 led to cross presentation and also Th1 responses [34]. The delivery of antigens to DEC205 has also been applied to tumor models, particularly melanoma and breast cancers. Additional studies employing recombinant chimeric antibodies, chemically-coupled conjugates, and scFv molecules have demonstrated that the delivery of tumor-associated antigens (such as tyrosinase-related protein 2) or other antigens known to be involved in tumorigenesis (such as survivin) to DEC205-expressing cells may lead to decreased tumor burden when such antigens are co-administered with adjuvants such as CpG, Poly(l:C), or anti-CD40. Tumor shrinkage is generally associated with enhanced immune responses, including antigen-specific $CD4^+$ and $CD8^+$ T cell responses and increases in pro-inflammatory cytokine production [35–41]. The capacity of anti-DEC205-mediated antigen targeting to increase T cell responses to infectious agents and tumors renders these antibodies central to the design of new vaccine-based immunotherapies [Figure 1].

Notably, especially under some pro-inflammatory conditions, expression of murine DEC205 is not limited to DCs. Upon administration of specific adjuvants such as alum *in vivo* or stimulation with IL-4, anti-CD40, or lipopolysaccharide (LPS) *in vitro*, germinal center B cells upregulate expression of DEC205. This upregulation of DEC205 by germinal center B cells may render antigen delivery to DEC205-expressing cells broader in scope and could serve as a vehicle for enhancing additional humoral responses [24, 42–46].

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Human DC1s also highly express DEC205, although an expression of human DEC205 is not limited to DC1s [47, 48]. Such broader expression of DEC205 on human cells may additionally account for the promising effects of treatments with anti-human(h)DEC205 chimeric antibodies. The *in vivo* administration of anti-hDEC205 antibodies fused to Epstein-Barr virus or human immunodeficiency (HIV) antigens together with the adjuvant Poly(l:C) elicits antiviral T cell responses [46, 49–51]. Moreover, cancer patients who were vaccinated with anti-hDEC205-NY-ESO-1 tumor antigen in conjunction with Toil-like receptor (TLR) agonists experienced increases in cellular and humoral immunity against the tumor, and some patients even experienced tumor regression [52, 53]. Given the successes of anti-hDEC205-Ag chimeric antibody administration, it is likely that additional pathogen-and tumor-derived antigens will be included in future vaccine strategies based on targeting through DEC205.

Additional Cell Surface Molecules Used For Antigen Targeting

The methodological and immune-modulating successes of targeting antigen via DEC205 prompted the introduction of antibodies targeting other molecules. These designs, which are produced via similar genetic techniques, employ corresponding V regions specific for other cell surface molecules present on DCs and pDCs [2, 23, 34, 54–59]. The CD11c integrin, which is expressed by murine DCs, has been frequently targeted with antigens by various strategies, demonstrating the capacity of anti-CD11c targeting to induce cellular and humoral responses *in vivo* [60–62]. More recently, the recombinant chimeric anti-CD11c-MOG antibody was produced based on the design containing murine lgG1 constant regions, as in the case of anti-DEC205 [54]. Importantly, as such anti-CD11c delivers antigens to all DCs irrespectively of subsets, the *in vivo* administration of this chimeric antibody in genetically modified mice that lack specific subsets of DCs can further advance the understanding of the functions of individual DC1 and DC2 subsets. For example, in the steady state, DC1s robustly induce pTreg cells, and such pTreg cells are crucial for ameliorating autoimmune responses such as those in EAE [63–65]. This induction of pTreg cells depends on functions of the BTLA molecule that is specifically expressed on some DC1s but not on DC2s [2, 12, 54]. In contrast, when antigens are delivered via anti-CD11c, only inefficient pTreg cell differentiation ensues because the majority of such antigen is presented by BTLA^{neg} DCs. However, in mice with a DC-specific deletion in IRF4, the DC1:DC2 ratio is increased, and pTreg cell differentiation is restored [2, 54]. Overall, the combination of genetic models with a targeted antigen delivery by recombinant chimeric antibodies can help clarify the specific functions of DC subsets including those applicable to human immunology [2, 7, 12, 14, 15, 54, 66, 67].

Many other antigen-targeting antibodies have been produced. Anti-Langerin-Ag [34, 55, 56], anti-Treml4-Ag [56, 57], and anti-Clec9a-Ag [68] have been produced and employed as alternatives to anti-DEC205-Ag to deliver antigen to DC1s, while anti-DCIR2-Ag delivers antigen to DC2s [23] and anti-Siglec-H-Ag [58] and anti-BST2-Ag [59] deliver antigen to pDCs. Though the cell-specific targeting of these antibodies may differ, their ability to enhance cognate T cell responses has been demonstrated in a wide variety of studies, some of which are discussed in greater detail below.

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Langerin (CD207) is a transmembrane protein that functions as an endocytic receptor by binding various sugars and certain pathogens such as HIV and Candida albicans [69–75]. It is expressed at relatively low levels on the cell surfaces of Langerhans cells and some $CD8a^+$ $DEC205^+$ DC1s of the spleen and skin draining lymph nodes in mice and at similarly-low levels on human lymphoid and tissue-resident DCs [67, 69–74, 76–80]. Anti-Langerin-Ag antibodies target antigen to $Langerin^+$ DCs of the spleen and peripheral lymph nodes *in vivo*, resulting in long-lasting antigen presentation on MHCI and MHCII molecules to $CD8^+$ and $CD4^+$ T cells, respectively. The targeting of $MOG_{35–55}$ peptide by anti-Langerin-MOG to skin $Langerin^+$ migratory DCs also lessened EAE symptom severity in a manner similar to that observed following administration of anti-DEC205-MOG [2, 55, 56].

A more recently discovered cell surface receptor, Trem-like 4 (Treml4), is a member of the "triggering receptor expressed on myeloid cells" family that binds apoptotic or necrotic cells. Though the expression of Treml4 in human DCs remains unclear, the expression of Treml4 in mice primarily occurs on $CD8a^+$ DCs and macrophages of the spleen [57, 81]. The targeting of diverse antigens to $Treml4^+$ DC1s by anti-Treml4 antibodies has been shown to elicit both $CD4^+$ and $CD8^+$ T cell responses; however, the effects of antigen delivery on disease severity in various models including tumor transplantation and EAE remain to be fully elucidated [56, 57].

C-type lectin domain family 9A, also known as DC NK lectin group receptor-1 (Clec9a, DNGR-1), is an endocytic C-type lectin receptor that binds necrotic cells and presents the processed antigens on MHCI and MHCII. It is primarily expressed by murine $CD8a^+$ DCs and pDCs and by human $BDCA3^+$ DCs, with additional low-level expression on human monocytes and B cells [82–89]. Under steady state conditions, antigen delivered to DCs via anti-Clec9a chemically-conjugated antibodies and presented on MHCII prompted the differentiation of $Foxp3^+$ T cells [68], Moreover, as antigen targeting to Poly(l:C)-matured DCs using anti-Clec9a-Ag antibodies results in Th1 $CD4^+$ and $CD8^+$ T cell priming further comparable to that observed following administration of anti-DEC205-Ag or anti-Langerin-Ag, the therapeutic capacities of these reagents may overlap under some immunological conditions, potentially providing additional options for immunotherapy [2, 34, 56, 57].

Currently, fewer options exist for targeting antigen to DCs of the DC2 lineage. The prime example of such an antibody is anti-DCIR2-Ag, which targets the DC inhibitory receptor 2 lectin. DCIR2 (also known as Clec4a4) is expressed by some murine $CD8^{neg}$ DC2s localized in the splenic marginal zone and red pulp, while Clec4a (DCIR), the sole human DCIR family member, is broadly expressed on myeloid DCs, pDCs, and other professional antigen presenting cells (APCs) [23, 90–95]. As demonstrated, altering the extracellular immune conditions during antigen delivery to DCIR2 or Clec4a may mount pro-immunogenic or tolerogenic $CD4^+$ T cell responses, leading to desirable outcomes such as prolonged survival in tumor models [23, 35, 94, 96].

Murine pDCs may also be specifically targeted with recombinant chimeric antibodies against molecules including sialic acid binding lg-like lectin H (Siglec-H) and bone marrow stromal cell antigen 2 (BST2), which correlate with human pDC-expressed molecules, to elicit pro-immunogenic or tolerogenic T cell responses [11, 58, 59, 97–101], Notably, under

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steady state conditions, targeting antigen to pDCs blocks autoimmune reactions, whereas such targeting may lead to antiviral and anti-tumor T cell responses in the presence of adjuvant [58, 59].

The targeted delivery of antigens through surface molecules such as DEC205 did not result in altered immune phenotypes *in vivo*, illustrating the absence of specific intrinsic signaling leading to changes in the DC's inherent functions initiated by a ligation of anti-DEC205-Ag [2, 20, 21]. In contrast, alterations in external immune conditions, such as those brought about by the addition of adjuvants, affected the balance of pro-immunogenic responses against and tolerogenic responses toward the delivered antigen [2, 20, 21], However, some other surface receptors used for antigen targeting, including DCIR2 and DCIR (Clec4a2), may exhibit intrinsic immunoregulatory signaling properties, when crosslinked by antibodies, such as the inhibition of IFNa production despite the presence of adjuvants [95, 102–104], Additionally, the engagement of the "lectin-like" scavenger receptor DC-asialoglycoprotein receptor (DC-ASGPR), whose expression is limited to human and non-human primate cells, can lead to increased production of IL-10 [105, 106], Moreover, a deficiency of Treml4, another receptor used for antigen targeting, has been associated with a decrease in pro-inflammatory cytokine production and autoimmunity-inducing antibodies, further indicating the potential plasticity in the regulatory or pro-immunogenic responses of certain cell surface molecules. It has not been established, though, whether such effects are directly correlated with antibody-mediated antigen delivery [57, 107], In contrast to the proposed inherent immunomodulatory properties of certain DC cell surface molecules, targeting to the Clec9a receptor has been shown to elicit potent humoral responses [82, 108–110], Similarly, the original anti-CD11c hamster or rat IgG and Fab fragments introduced to mice could boost the humoral response to the antigen [62], However, targeting of antigen to mouse CD11c by a recombinant chimeric antibody containing murine constant regions is not immunogenic [54].

As stated previously, recombinant chimeric antibodies may generally be a superior choice for antigen targeting purposes because they are modified in a manner that reduces off-target immune effects. The use of recombinant chimeric antibodies containing mutated, species-matched constant regions minimizes the possibility of non-specific cross-linking *in vivo*. Further, studies combining murine genetic models lacking specific subsets of DCs and the *in vivo* targeting of antigens to multiple cell surface molecules can provide additional confirmation that the observed results represent the intrinsic functions of DCs unaffected by the signaling elicited by ligation of the surface molecule used for targeting.

Conclusions

In conclusion, targeting antigens, particularly to dendritic cells and by using recombinant chimeric antibodies, has become an important tool in both basic science and clinical applications. Though the expression of specific cell surface molecules may differ between murine and human DCs, the conservation of DCs and their subsets provides scientific justification for further studies in animal models of disease as well as for additional expansion of such applications in the immunotherapeutic contexts.

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Highlights:

- Recombinant chimeric antibodies faithfully deliver antigens in vivo.
- Targeted antigen delivery achieves specific immunomodulation.
- DEC205 remains an important target for therapeutic antigen delivery.

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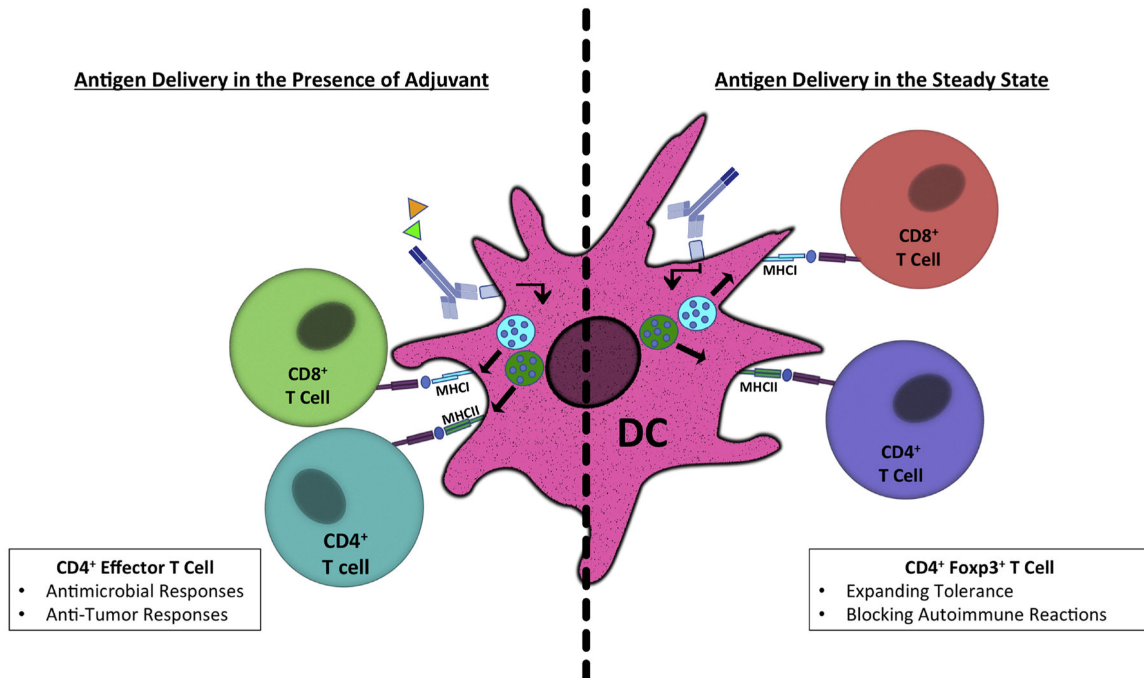

Figure 1: Administration of antigen-targeting antibodies to DCs directs antigen-specific T cell responses.

The delivery of antigens (Ag) to DCs or their subsets results in the processing and presentation of antigenic peptides on MHCII to $CD4^+$ T cells as well as in the crosspresentation of such peptides on MHCI to $CD8^+$ T cells [2, 8–10, 23, 24, 54], In the absence of pro-immunogenic stimuli (“steady state”), DCs induce a deletion and other forms of T cell tolerance including a conversion of $CD4^+$ T cells to Ag-specific peripheral regulatory T (pTreg) cells [20, 21], In the presence of pro-immunogenic stimuli such as the adjuvants Poly(l:C), CpG, or anti-CD40 (represented as triangles), DCs can induce Ag-specific effector and cytotoxic T cells [32–41].